Estradiol Regulation of Hypothalamic Astrocyte Glycogen

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• ULM is a multi-purpose, state-assisted institution of higher education which offers high quality academic and experiential opportunities to meet the academic, cultural, vocational, social, and personal needs of undergraduate, graduate, and continuing education students.

• ULM claims more than 65,000 alumni and an annual enrollment of approximately 9,200 students.

• The university is a member of the University of Louisiana System, which is comprised of nine universities throughout Louisiana.

• ULM is the hub for healthcare and pharmacy training and research for northeast Louisiana.
BPS Department Mission:
Advance health care through
• Cutting-edge research and instruction on
  • the nature and utilization of chemicals as medicines
  • chemical interactions with biological systems
  • optimal modes of delivery of therapeutic agents
• Preparation of capable and proficient students for productive professional and research careers in the pharmaceutical sciences.
• Scholarly activity spans traditional disciplinary boundaries, integrating bio- and analytical chemistry, anatomy, physiology, and molecular biology in studies on interaction of chemicals with biological systems.

• BPS Research is focuses on novel nanoparticle-based drug-delivery systems; mammary, prostate, and pituitary tumorigenesis; reproductive neuroendocrinology and energy homeostasis; and marine natural products chemistry.

• Extramural funding from national, state, and private funding sources, including the National Institutes of Health (NIH), National Science Foundation, American Heart Association, American Diabetes Association, and Juvenile Diabetes Research Foundation.
• Research training involves rigorous foundational preparation, mastery of practical and advanced laboratory skills, and access to critical, cutting-edge instrumentation and technologies.

• BPS graduate trainees are mentored with the goal of conducting innovative, creative, hypothesis-driven research that addresses critical societal health concerns, using modern approaches.

• BPS’ interdisciplinary culture fosters and promotes interaction and collaboration among scientists with diverse research training and experience, and provides students with this important perspective.
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NIH; National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)

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09/19/2016 – 07/31/2021

$1,740,000.00 Total Costs

Collaborative initiative involving University of North Texas Dept. of Physics IMBAL facility
Pre-Award Activities:

- Selection of Funding Source
- Time Frame for Proposal Preparation
  Proposal Narrative (Research Goals, Plan)
  Budget and Justification
  Biosketches, Letters of Collaboration, etc.
- Institutional Registrations
- OSPR
- Personal Submission History
- Program Officer Engagement
Abbreviations:
AR: Adrenergic receptor
ER: Estradiol receptor
Glyc: Glycogen
VMN: Ventromedial hypothalamic nucleus
Abstract:

(Need) Glycogen is a critical reserve of oxidizable fuel for brain neuron use during states of heightened activity or glucoprivation. Iatrogenic hypoglycemia is a recurring complication of obligatory meticulous control of insulin-dependent diabetes mellitus and poses a serious risk of neural injury. There is thus keen interest to maximize glycogen protection against hypoglycemic harm.

(Premise) This project will address the hypothesis that estradiol controls hypothalamic glycogen metabolism by regulation of astrocyte metabolic sensor function, and that estrogen augmentation of the glycogen fuel reserve correlates with prolonged local nerve cell energetic stability during hypoglycemia.
(Plans)
1) Determine effects of estradiol on hypothalamic glycogen content in normo- and hypoglycemic female and male rats
2) Assess the role of astrocyte estrogen receptor variants in energy sensor-mediated glycogen metabolic responses to glucoprivation in each sex
3) Determine how hindbrain norepinephrine and estrogen interact to optimize hypothalamic astrocyte glycogen metabolism.

(Tools)
1) Immunocytochemistry/laser-capture microdissection/high-sensitivity qPCR and Western blotting; 2) Pharmacologic agents for receptor manipulation; 3) Nano-technological devices for astrocyte-specific gene silencing; 4) Glycogen densitometric histochemistry and mass spectrometry; 5) High-energy focused ion beam technology for in situ high-resolution mapping of macro- and microminerals in brain
(Anticipated Outcomes): This work will utilize an innovative assembly of investigative strategies to identify cellular and molecular mechanisms of estrogen regulation of astrocyte glycogen mass, knowledge that can be leveraged to advance development of therapeutic strategies for neuro-protective amplification of brain glycogen levels.
In the clinical setting, insulin-induced hypoglycemia is a recurring complication of obligatory meticulous control of type I (insulin-dependent) diabetes mellitus. Enhanced astrocyte glycogen mass alleviates the risk of neuron dysfunction and demise associated with this condition.

This project will advance the mission of the NIH through identification of mechanisms to maximize brain glycogen deterrence of harm by hypoglycemia.
Specific Aims:

Astrocytes promote neuro-metabolic stability through uptake, storage, and catabolism of glucose. These glial cells maintain the brain’s major glycolytic reservoir, which functions as a critical metabolic fuel supply to neurons during glucose privation. Iatrogenic insulin-induced hypoglycemia is a recurring complication of obligatory meticulous control of insulin-dependent diabetes mellitus. As vital neuron functions are maintained at high energy expense, hypoglycemia-associated reductions in glucose supply to the brain pose a serious risk of nerve cell injury. Enhanced astrocyte glycolysis mass alleviates neuron dysfunction and demise during energy deficiency, including in vivo hypoglycemia [Swanson and Choi, 1993; Wende et al., 2000; Brown et al., 2004; Suh et al., 2007]. There is thus strong motivation to develop therapeutic tools to maximize neuro-protection afforded by the brain glycolysis reserve.

The hypothalamus provides sensory information on cellular metabolic state to the brain-wide glyco-regulatory network. Distinct hypothalamic loci, including the ventromedial hypothalamic nucleus (VMH), contain specialized substrate fuel-sensing neurons that alter synaptic firing during hypoglycemia [Ashford et al., 1990; Silver and Erecitska, 1998] to translate alterations in energy balance into neural signaling. There is growing recognition of neural and endocrine regulation of astrocyte glycolysis metabolism. Noradrenaline stimulates glycolysis breakdown in cerebral cortex in vitro [Harik et al., 1982] and cultured cortical astrocytes in vitro [Pellegrin et al., 1997]. Glycolysis metabolism is regulated by opposing actions of glycogen synthase (GS) and glycogen phosphorylase (GP), which respectively catalyze glycolysis synthesis and depletion. Our studies show that the ovarian steroid 17β-estradiol respectively increases and decreases basal VMH astrocyte GS and GP protein expression, but augments GP content in these glia during hypoglycemia; moreover, our data indicate that these actions are hindbrain catecholamine-dependent [Tamrakar et al., 2015]. These results underscore the need for insight on estradiol regulation of VMH glycolytic content and turnover during glucose sufficiency and shortage in each sex. This project will integrate an array of approaches, including single-cell laser-microdissection, high-sensitivity q-RT-PCR and Western blot techniques, pharmacological tools to up- or down-regulate VMH astrocyte glycolysis breakdown during hypo- versus normoglycemic conditions, cell type-specific gene knockdown nanotechnology, and high-resolution quantitative elemental microanalysis, to address the overarching hypothesis that estradiol regulates VMH glycolytic content through receptor variant-mediated control of astrocyte glycolysis metabolism, and that sex-specific estrogen augmentation of this fuel reserve correlates with divergent patterns of metabolic-sensory nerve cell signaling of energetic stability during in vivo hypoglycemia in each sex.

This central hypothesis will be addressed by the following Specific Aims:

- **Specific Aim 1:** Determine effects of estradiol on VMH glycolytic content and associated impact on nerve cell energy state during hypoglycemia in female and male rats. This Aim will address the premise that augmentation of VMH glycolytic levels by estradiol differs between the sexes, and correlates with sex-specific preservation of VMH nerve cell energy stability and adjustments in electrolytic indices of electrical activity in VMH metabolic sensory neurons during hypoglycemia.

- **Specific Aim 2:** Assess estrogen receptor role and molecular mechanisms of action in regulation of glycolysis metabolism during glucose sufficiency and shortage. VMH astrocyte adenosine 5’-monophosphate-activated protein kinase (AMPK) activity is augmented during hypoglycemia through estradiol-dependent mechanisms. AMPK is critical for hypoglycemic up-regulation of GP. This Aim will use established primary VMH astrocyte cultures as a model to characterize ER variants and post-receptor nuclear- versus membrane-initiated mechanisms that regulate glycolysis metabolism and mass via AMPK.

- **Specific Aim 3:** Determine molecular substrates for Hindbrain noradrenergic and estrogen interaction in controlling VMH astrocyte glycolysis metabolism. This Aim will address the working premise that ER variant regulation of noradrenaline (NE) augmentation of glycolytic patterns of VMH astrocyte AMPK activity is crucial for sex-specific patterns of glycolysis mobilization during hypoglycemia. This work will also examine whether AMPK control of glycolysis metabolism involves estradiol regulation of noradrenergic- and hypoglycemic patterns of A2 noradrenergic signaling to and/or VMH astrocyte receptivity to NE unique to each sex.

**Anticipated Research Outcomes:** This project is significant for bringing focus to sex-specific effects of estradiol on brain fuel reserves and nerve cell energy state during hypoglycemia. The cohesive assembly of innovative investigative techniques outlined here is expected to identify cellular and molecular mechanisms of estrogen control of astrocyte glycolysis metabolism, knowledge that can be leveraged to advance development of therapeutic strategies for neuro-protective amplification of brain glycolysis levels in each sex.
Research Plan

• **Significance**
  Human health context, statement of need, Hypothesis, Anticipated outcomes/benefits

• **Innovation**
  Conceptual, technological

• **Approach**
  Preliminary Data, Experimental Design
  (treatment groups, animal numbers, chronological schedule of work, analytical procedures, statistics), Equipment

• **Investigator** *(Biosketch w/personal statement and NCBI bibliography link)*

• **Environment**
**Application Strengths:**

- Incorporation of sex comparisons to determine biological differences in hypothalamic glycogen regulation during eu- and hypoglycemia

- Array of diverse, complimentary state-of-the-art analytical approaches

- Molecular-, cellular-, systemic-level investigative focus

- Interdisciplinary approach involving neuroscience, molecular/cellular biological, pharmaceutics, and applied physics expertise